On page 47, immediately preceding the claims, please insert the enclosed text entitled "SEQUENCE LISTING".

REMARKS

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made."

These amendments are made in adherence with 37 C.F.R. § 1.821-1.825. This amendment is accompanied by a floppy disk containing the above named sequence, SEQUENCE ID NUMBERS 1-19, in computer readable form, and a paper copy of the sequence information. The computer readable sequence listing was prepared through use of the software program "Patent-In" provided by the PTO. The information contained in the computer readable disk is identical to that of the paper copy. This amendment contains no new matter. Applicant submits that this amendment, the accompanying computer readable sequence listing, and the paper copy thereof serve to place this application in a condition of adherence to the rules 37 C.F.R. § 1.821-1.825.

The Commissioner is authorized to charge any fees, including extension fees, which may be required, or credit any overpayment to Deposit Account No. 06-1300 (Our Order No. A-70036/RMS/JJD).

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Please direct any calls in connection with this application to the undersigned at (415) 781-1989.

Respectfully submitted,

FLEHR HOHBACH TEST
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Dated: June 7, 2001

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION

Paragraph beginning at page 4, line 14, has been amended as follows:

– Figures 1A and 1B depict the germline ϵ locus and sequence. Fig. 1A (SEQ ID NO:1) depicts the sequence of the human IL-4 inducible ϵ promoter. Fig. 1B depicts the organization of the germline ϵ locus. –

Paragraph beginning at page 4, line 17, has been amended as follows:

– Figures ~~2A and~~ 2A, 2B and 2Cdepict the regions (2A) and sequences (2B and 2C; SEQ ID NOS:2 and 3) of the switch ϵ (Sϵ) region that are used in methods of screening for proteins that interact with the Sϵ region, as described below. –

Paragraph beginning at page 5, line 28, has been amended as follows:

– Figures 11A, 11B and 11C (SEQ ID NOS:4, 5 and 6) depict preferred vectors and their sequences.–

Paragraph beginning at page 5, line 30, has been amended as follows:

– Figures 12A, 12B and 12C (SEQ ID NO:7) depict a construct useful in the present invention, comprising the a Fas survival construct (i.e. the use of a death gene). The sequence is of the inducible ϵ promoter-chimeric Fas-IRES-hygromycin-bovine growth hormone poly A tail that is put into the C12s vector backwards to that no leaky transcription happens through the cmv promoter. –

Paragraph beginning at page 5, line 35, has been amended as follows:

– Figures 13A, 13B and 13C (SEQ ID NO:8) depict a construct useful in the present invention, comprising the a Fas survival construct (i.e. the use of a death gene). The sequence is of the inducible ε promoter-chimeric Fas (either CD8 or mLyt2)-IRES-hygromycin-bovine growth hormone poly A tail that is put into the C12s vector backwards to that no leaky transcription happens through the cmv promoter. –

Paragraph beginning at page 7, line 5, has been amended as follows:

– In a preferred embodiment, the invention provides methods of screening for bioactive agents capable of modulating, particularly inhibiting, an IL-4 inducible ε promoter. By "an IL-4 inducible promoter" herein is meant a nucleic acid promoter that is induced by IL-4, putatively by binding an unknown IL-4 induced DNA binding protein that results in induction of the promoter; that is, the introduction of IL-4 causes the pronounced activation of a particular DNA binding protein that then binds to the IL-4 inducible promoter segment and induces transcription. The sequence of the human IL-4 inducible promoter is shown in Figure ~~1~~ 1A (SEQ ID NO:1), and as will be appreciated by those in the art, derivatives or mutant promoters are included within this definition. Particularly included within the definition of an IL-4 inducible promoter are fragments or deletions of the sequence shown in Figure ~~1~~ 1A (SEQ ID NO:1). As is known in the art, the IL-4 inducible promoter is also inducible by IL-13. By "modulating an IL-4 inducible promoter" herein is meant either an increase or a decrease (inhibition) of promoter activity, for example as measured by the presence or quantification of transcripts or of translation products. By "inhibiting an IL-4 inducible promoter" herein is meant a decrease in promoter activity, with changes of at least about 50% being preferred, and at least about 90% being particularly preferred. –

Paragraph beginning at page 24, line 26, has been amended as follows:

– A preferred coiled-coil presentation structure is as follows:

MGCAALESEVSALESEVASLESEVAAL**GRGDMP**LAAVKSKLSAVKSKLASVKSKLAACGPP (SEQ ID NO:9). The underlined regions represent a coiled-coil leucine zipper region defined previously (see Martin et al., EMBO J. 13(22):5303-5309 (1994), incorporated by reference). The bolded GRGDMP (SEQ ID NO:10) region represents the loop structure and when appropriately replaced with randomized peptides (i.e.candidate bioactive agents, generally depicted herein as $(X)_n$, where X is an amino acid residue and n is an integer of at least 5 or 6) can be of variable length. The replacement of the bolded region is facilitated by encoding restriction endonuclease sites in the underlined regions, which allows the direct incorporation of randomized oligonucleotides at these positions. For example, a preferred embodiment generates a XhoI site at the double underlined LE site and a HindIII site at the double-underlined KL site. –

Paragraph beginning at page 25, line 10, has been amended as follows:

– A preferred minibody presentation structure is as follows: MGRNSQATS**GFT***F***SHF**YMEWVRGGEYIAASR**HKHNKY**TTEYSASVKGRYIVSRDTSQSILYLQ KKKGPP (SEQ ID NO:11). The bold, underline regions are the regions which may be randomized. The ~~italized~~ italicized phenylalanine must be invariant in the first randomizing region. The entire peptide is cloned in a three-oligonucleotide variation of the coiled-coil embodiment, thus allowing two different randomizing regions to be incorporated simultaneously. This embodiment utilizes non-palindromic BstXI sites on the termini.–

Paragraph beginning at page 26, line 1, has been amended as follows:

– In a preferred embodiment, the targeting sequence is a nuclear localization signal (NLS). ~~NLSs~~ NLSes are generally short, positively charged (basic) domains that serve to direct the entire protein

in which they occur to the cell's nucleus. Numerous NLS amino acid sequences have been reported including single basic ~~NLS's~~ NLSes such as that of the SV40 (monkey virus) large T Antigen (Pro Lys Lys Lys Arg Lys Val (SEQ ID NO:12)), Kalderon (1984), et al., Cell, 39:499-509; the human retinoic acid receptor-ß nuclear localization signal (ARRRRP (SEQ ID NO:13)); NFκB p50 (EEVQRKRQKL (SEQ ID NO:14); Ghosh et al., Cell 62:1019 (1990)); NFκB p65 (EEKRKRTYE (SEQ ID NO:15); Nolan et al., Cell 64:961 (1991)); and others (see for example Boulikas, J. Cell. Biochem. 55(1):32-58 (1994), hereby incorporated by reference) and double basic ~~NLS's~~ NLSes exemplified by that of the Xenopus (African clawed toad) protein, nucleoplasmin (Ala Val Lys Arg Pro Ala Ala Thr Lys Lys Ala Gly Gln Ala Lys Lys Lys Lys Leu Asp (SEQ ID NO:16), Dingwall, et al., Cell, 30:449-458, 1982 and Dingwall, et al., J. Cell Biol., 107:641-849; 1988). Numerous localization studies have demonstrated that ~~NLSs~~ NLSes incorporated in synthetic peptides or grafted onto reporter proteins not normally targeted to the cell nucleus cause these peptides and reporter proteins to be concentrated in the nucleus. See, for example, Dingwall, and Laskey, Ann, Rev. Cell Biol., 2:367-390, 1986; Bonnerot, et al., Proc. Natl. Acad. Sci. USA, 84:6795-6799, 1987; Galileo, et al., Proc. Natl. Acad. Sci. USA, 87:458-462, 1990.–

Paragraph beginning at page 26, line 29, has been amended as follows:

– In a preferred embodiment, the fusion partner is a stability sequence to confer stability to the candidate bioactive agent or the nucleic acid encoding it. Thus, for example, peptides may be stabilized by the incorporation of glycines after the initiation methionine (MG or MGG0), for protection of the peptide to ubiquitination as per Varshavsky's N-End Rule, thus conferring long half-life in the cytoplasm. Similarly, two prolines at the C-terminus impart peptides that are largely resistant to carboxypeptidase action. The presence of two glycines prior to the prolines impart both flexibility and prevent structure initiating events in the di-proline to be propagated into the candidate peptide structure. Thus, preferred stability sequences are as follows: $MG(X)_nGGPP$ (SEQ ID NO:17), where X is any amino acid and n is an integer of at least four. –

Paragraph beginning at page 28, line 4, has been amended as follows:

— In a preferred embodiment, the fusion partner includes a linker or tethering sequence, as generally described in PCT US 97/01019, that can allow the candidate agents to interact with potential targets unhindered. For example, when the candidate bioactive agent is a peptide, useful linkers include glycine-serine polymers (including, for example, $(GS)_n$, $(GSGGS)_n$ (SEQ ID NO:18) and $(GGGS)_n$ (SEQ ID NO:19), where n is an integer of at least one), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers such as the tether for the shaker potassium channel, and a large variety of other flexible linkers, as will be appreciated by those in the art. Glycine-serine polymers are preferred since both of these amino acids are relatively unstructured, and therefore may be able to serve as a neutral tether between components. Secondly, serine is hydrophilic and therefore able to solubilize what could be a globular glycine chain. Third, similar chains have been shown to be effective in joining subunits of recombinant proteins such as single chain antibodies. —

On page 47, immediately preceding the claims, the enclosed text entitled "SEQUENCE LISTING" was inserted into the text.